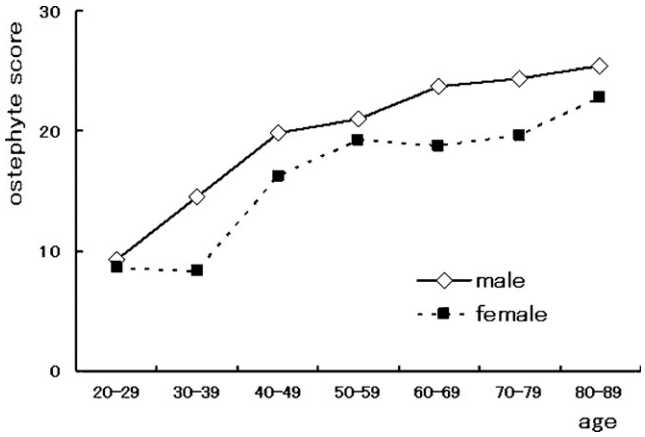
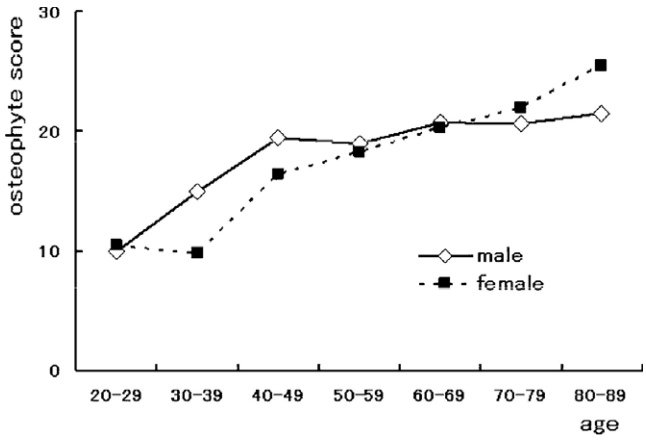
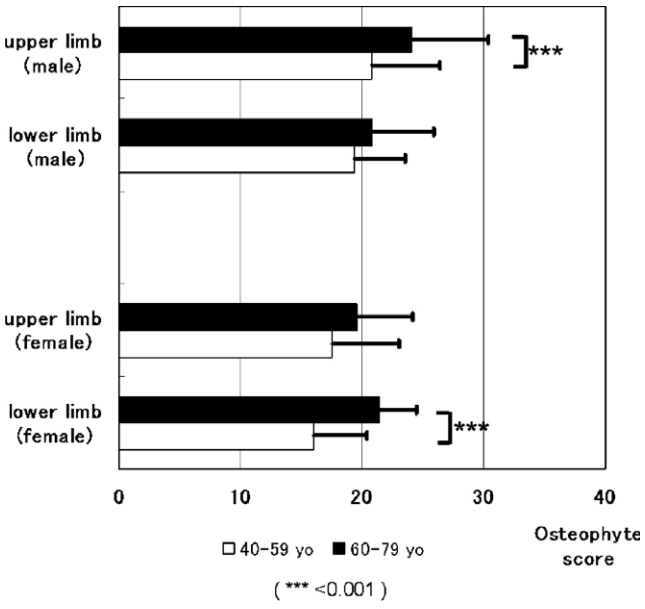
and lower extremities were observed and scored in order to analyze their distributions and investigate their biological and clinical significance.
Methods: Three hundred and seventy Japanese skeletons (male 232 and female 138) were examined. All of the six major joints, shoulder, elbow, wrist, hip, knee and ankle were observed macroscopically. Marginal areas of each joint surface were divided into regions to evaluate the degree of peri-articular osteophytes in each region. These osteophytes were graded 0, 1, 2, 3 or 4 based on our criteria, and the scores of each joint were calculated.







Results: With the exception of the ankle in males, all scores of the major six joints were correlated with age; most scores showed a strong correlation coefficient less than 0.001. There were some gender differences; for example, in the shoulder joint, the scores of males exceeded those of females at all ages (Figure 1); conversely in the knee joint, the scores for females were smaller than those of males until the fifties, then increased to become higher than those of males after sixty years old (Figure 2). To clarify the pattern of increase in the scores of each joint, average scores for forty- and fifty-year-old groups (middle-age group) were compared with those of the sixty- and seventy-year-old groups (elderly group). In males, the shoulder and the wrist showed significant differences between these two age groups; however, in females, the three joints of the lower extremity, hip, knee and ankle joints showed significant differences. Then, the average scores of the upper extremities (shoulder, elbow and wrist joints) and those of the lower extremities (hip, knee and ankle joints) were calculated to compare these two groups. In males, there was a significant increase of the scores only in the upper limbs; conversely in female, there was a significant increase only in the lower limbs (Figure 3).
Conclusions: In skeletons of vertebrates such as humans, synovial joints with articular cartilage on their surfaces have developed in order to gain greater flexibility. Articular cartilage is weaker than bone, and therefore their surface areas become enlarged to decrease the stress of mechanical load. Osteophyte formation seems to link these biological rationalities. It is known that these osteophytes proliferate extensively under osteoarthritic condition; however, this study showed that the osteophyte scores began to increase in the middle-aged population before the onset of OA conditions. The development of these osteophyte formations is affected by various factors: systematic condition and/or local environment. In addition, this study reassessed the potent influence of the unequal distribution of mechanical stress on joint surfaces in the facilitation of osteophyte formation and OA pathogenesis.

132 CCL20 CHEMOKINE INDUCE DIFFERENTIAL EFFECTS ON PROLIFERATION AND AKT SIGNALING IN HUMAN OSTEOBLASTS FROM OSTEOARTHRITIS COMPARED TO RHEUMATOID ARTHRITIS PATIENTS

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Purpose: Osteoarthritis (OA) and rheumatoid arthritis (RA) affect not only cartilage but also the entire joint structure. In OA joint, bone changes are characterized by the formation of new bone (osteophytes) and fibrosis while in RA a progressive bone and joint destruction leads to joint instability. We previously demonstrated that CCL20 chemokine induced both osteoblast proliferation and osteoclast differentiation. This study aimed to evaluate the expression of CCR6-CCL20 receptor-chemokine expression on osteoblasts isolated from OA and RA patients and on bone tissue biopsies. Then we analyzed CCL20 chemokine signaling on osteoblasts from patients of both groups.
Methods: CCR6 was analysed by flow cytometry on isolated osteoblasts while CCL20 release was evaluated by ELISA. Different functional assays (β-N-acetylhexosaminidase release, α-actin expression and cell proliferation) were performed to assess the functional role of CCL20. Moreover, Akt and Erk1/2 signaling pathways were also analyzed at the protein level by Bio-plex assay. CCR6, CCL20 and Akt expression were also tested by immunohistochemistry in bone tissue biopsies of OA and RA patients.
Results: Flow cytometric analysis of CCR6 receptor on osteoblasts and immunohistochemical evaluation in bone tissue biopsies of OA and RA patients evidenced a high expression of this marker. By contrast, CCL20 was not released in basal condition by osteoblasts from OA and RA patients but only after stimulation with IL1β alone and in combination with TNFα. Immunohistochemical analysis of CCL20 in bone tissue biopsies demonstrated that was highly expressed only in osteoblasts, osteocytes and infiltrating mononuclear cells in RA patients. By contrast, in OA biopsies CCL20 expression was limited to a few cases that showed limited areas of bone remodeling or the presence of infiltrating mononuclear cells. Functional assays demonstrated that both β-N-acetylhexosaminidase release and α-actin expression were induced by CCL20 in osteoblasts from OA and RA patients. Moreover, CCL20 chemokine significantly induced osteoblasts proliferation in RA patients but not in OA patients. CCL20 did not induced Erk1/2 signaling pathway but we observed an increase of Akt signaling only in osteoblasts from RA patients. Immunohistochemical analysis of Akt expression on bone tissue biopsies confirmed an increase expression of this marker in RA biopsies.
Conclusions: This study demonstrated differential effects of CCL20 chemokine in osteoblasts from OA and RA patients and these data were

confirmed on bone tissue biopsies clearly indicating that this chemokine is influenced by the bone milieu and by the evolution of the disease.

133 MODULATION OF THE SYNTHESIS OF OSTEPROTEGERIN AND RECEPTOR ACTIVATOR OF THE NF-KB LIGAND IN THE SUBCHONDRAL BONE OF RABBITS WITH OSTEOPOROSIS AND OSTEOARTHRITIS

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Purpose: We have previously found that prior subchondral osteoporosis (OP) increases the severity of cartilage damage in an experimental model of osteoarthritis (OA) in rabbits. The aim of this study was to determine the changes in the synthesis of osteoprotegerin (OPG) and receptor activator of the NF-kB ligand (RANKL) in the subchondral bone of rabbits with a combined model of OP and OA. We also compared these changes with the bone mineral density (BMD) and the serum biochemical markers of bone turnover.
Methods: Eighteen NZW rabbits (8 months old) underwent bilateral OVX and subsequent parenteral methylprednisolone administration (1 mg/kg/d) for 4 weeks to induce OP (OP group). Nine age and gender matched animals were used as controls (Control group). OA was simultaneously induced by anterior cruciate ligament transection and partial medial meniscectomy in the left knees of all the rabbits. 22 weeks after OVX surgery, all the animals were sacrificed and then the left knees were considered osteoarthritic (OA) or osteoarthritic plus osteoporotic (OPOA), and the right knees were used as OP or healthy controls, respectively. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA) at baseline and 22 weeks after OVX (Hologic ® QDR-1000) at lumbar spine (L3-L4, LS), global knee (gK) and subchondral bone of the knee (sK). At sacrifice, we isolated total proteins from the subchondral bone of the knees in order to evaluate OPG and RANKL protein synthesis by western-blot. Serum concentrations of alkaline phosphatase (AP) and tartrate-resistant acid phosphatase (TRAP) were measured by enzymatic assays.
Results: At earlier as 6 weeks after OVX, OP rabbits showed a significant decrease in BMD, both at LS, gK and also at sK ($p < 0.05$ vs. controls at the three locations) that was maintained during all the period of study. At sacrifice, subchondral bone of OP rabbis showed a significant decrease in BMD when compared to controls (0.54 ± 0.02 vs. 0.66 ± 0.02 mg/cm^2, $p < 0.001$). At this time of study, serum TRAP concentration was significantly higher in OP rabbits when compared to controls (106 ± 6 vs. 59 ± 3 mM, $p = 0.003$), while no differences were found in serum AP concentration between these groups (25 ± 3 vs. 27 ± 3 mM, p = NS). Subchondral bone OPG synthesis was significantly decreased both in OP and in OPOA knees when compared to healthy controls (Controls: 1.0 ± 0.1; OP: $0.54 \pm 0.0.3$*; OA: 0.78 ± 0.07; OPOA 0.56 ± 0.02* arbitrary units; *$p < 0.05$ vs. controls), while RANKL protein synthesis only increased in OPOA when compared to controls (Controls: 1.0 ± 0.14; OP: 1.32 ± 0.09; OA: 1.4 ± 0.34; OPOA: 1.87 ± 0.14*, arbitrary units; *$p < 0.05$ vs. controls). Thus, OPG/RANKL ratio was significantly higher both in OP and in OPOA when compared to controls. OPG/RANKL ratio was also significantly higher in OPOA rabbits when compared both with OA and with OP groups. Moreover, we found a statistically significant positive correlation between OPG/RANKL protein synthesis ratio and subchondral bone BMD.
Conclusions: Our data showed that the decrease in the OPG/RANKL ratio is associated to an increased bone resorption and a decreased bone density in the subchondral bone of osteoporotic rabbits. Our data support the hypothesis that the increase in the subchondral bone remodelling might trigger articular cartilage damage.

134 SUBCHONDRAL BONE ATTRITION IS A REFLECTION OF COMPARTMENT-SPECIFIC MECHANICAL LOAD: THE MOST STUDY

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Purpose: While subchondral bone is thought to have an important role in OA progression, subchondral bone attrition (SBA) as detected by x-rays was traditionally thought to be a late finding in OA. However, SBA can be seen on MRI even in early OA, and may be related to mechanical and systemic factors which can contribute to altered properties of subchondral bone and OA progression. For example, focal loading related to malalignment, or presence of low bone density may predispose to SBA. We evaluated the effects of malalignment as a determinant of load across the knee and bone density as a systemic indicator of bone strength on the presence and incidence of SBA.
Methods: The Multicenter Osteoarthritis (MOST) Study is a NIH-funded longitudinal observational study of individuals who have or are at high risk for knee OA. Alignment was measured on baseline PA long-limb films at the knee and categorized as neutral (179–181º), varus (181º). BMD of the right femoral neck (g/cm^2) was assessed (Hologic 4500A) at baseline and categorized into age- and sex-specific tertiles. At baseline and 30-month follow-up, participants had knee MRIs performed (1.0 T; axial and sagittal proton density fat suppressed and coronal STIR sequences). MRIs were graded using WORMS in 5 subregions within each of the medial and lateral tibiofemoral (TF) compartments (central, posterior femur; anterior, central, posterior tibia) for SBA (0−3). A knee was eligible for the incident SBA analysis if SBA score = 0 in all subregions within a compartment at baseline. We evaluated the association of alignment and BMD with baseline presence of SBA (score $\geqslant$1 in any subregion within the compartment of interest) and incident SBA (any score increase from 0 at baseline in any subregion within the compartment of interest) using logistic regression for the medial and lateral TF compartments separately. GEE was used to account for correlations between knees within a subject. All analyses were adjusted for age, sex, and BMI, and analyses including BMD were additionally adjusted for bone-modulating medication use.
Results: There were 999 participants (1063 knees) with measures available for analyses (mean age 63, mean BMI 30.2, 63% female). Baseline SBA was present in 36%, and 50% had knee OA. Results in the Table demonstrate an association between presence of and incident SBA with varus alignment in the medial compartment, and with valgus alignment in the lateral compartment. Low BMD was not associated with SBA.

Table 1: Association of alignment and BMD with baseline presence of subchondral bone attrition, and with incident subchondral bone attrition over 30 months

	Adjusted ORs (95% CI)			
	Baseline presence of SBA		Incident SBA	
	Medial compartment (1063 knees)	Lateral compartment (1063 knees)	Medial compartment (706 knees)	Lateral compartment (872 knees)
Alignment[1]*				
Neutral (30%)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Varus (50%)	3.2 (2.2−4.6)	0.5(0.3−0.9)	1.9 (1.0−3.6)	0.4 (0.1−1.1)
Valgus (20%)	0.5 (0.3−0.9)	4.0 (2.5−6.6)	0.5 (0.2−1.2)	1.9 (0.7−5.1)
BMD2**				
Lowest tertile	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref
Middle tertile	1.2 (0.8−1.7)	1.5 (0.9−2.5)	1.4 (0.7−2.8)	1.5 (0.4−5.7)
Highest tertile	1.1 (0.7−1.4)	1.4 (0.8−2.3)	1.7 (0.9−3.5)	4.7 (1.5−15.1)

[1]Adjusted for age, sex, BMI; [2]Adjusted for age, sex, BMI, use of bone-modulating agents; *Alignment categories as follows: Neutral = 179–181º, Varus $\leqslant$ 179º, Valgus $\geqslant$ 181º; **BMD categorized into age- and sex-specific tertiles based on femoral neck BMD.

Conclusions: Presence and incidence of SBA are associated with malalignment, but not lower bone density. SBA is thus likely a marker of increased load experienced by overlying cartilage, and may in turn contribute to increased forces transmitted to the cartilage due to altered properties of subchondral bone related to SBA.

135 SYNERGISTIC EFFECT OF HYALURONAN AND INTERMITTENT HYDROSTATIC PRESSURE ON OSTEOBLASTS FROM OSTEOARTHRITIC SUBCHONDRAL BONE

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Purpose: The objective of the present study was to investigate the effects of intraarticular injection (IAI) of hyaluronan (hyaluronic acid, HA) and mechanical stress on osteoblasts isolated from osteoarthritic subchondral bone.
Methods: *Exp.1*; Osteoarthritis (OA) was induced in the left knee joints of Japanese white rabbits (3 kg) by performing transection of anterior cruciate ligament (ACLT). One week after the ACLT, fluorescent labeled HA (0.3 mg/0.3 ml) was administrated into the knee joints. The femur and tibia were resected from the joints a week after the administration. Samples were fixed in 4% paraformaldehyde and decalcified with Kalkitox™. Cryosections were observed with a fluorescence microscope. *Exp. 2*; OA